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Antioxidant Activity of Methanol Extract of *Tinospora crispa* and *Tabernaemontana corymbosa*

(Aktiviti Antioksida Ekstrak Metanol *Tinospora crispa* dan *Tabernaemontana corymbosa*)

HEIDA NADIA ZULKEFLI, JAMALUDIN MOHAMAD* & NURHAYATI ZAINAL ABIDIN

ABSTRACT

Tinospora crispa *and* Tabernaemontana corymbosa *have been used traditionally to treat fever, diabetes, rheumatism and sinusitis. The objective of this study was to evaluate the antioxidant activity of* Tinospora crispa *and* Tabernaemontana corymbosa. *The presence of apigenin and magnoflorine was detected using LCMS/MS in* Tinospora crispa *(Patawali) whereas appararicine, voafinine, conodusarine, conodurine, voacamine and voacangine were detected in* Tabernaemontana corymbosa *(Susur kelapa) methanol extract. The stem extract of* Tinospora crispa *showed high antioxidant activity in the following order: DPPH radical scavenging, reducing power and metal chelating assay (98.8%, 0.957, 81.97%) than* Tabernaemontana corymbosa *of leaves (90.04%, 0.652, 69.64%), stem (82.78%, 0.819, 36.70%) and root extracts (63.25%, 0.469, 51.56%), respectively. The high antioxidant activity in the stem extract of* Tinospora crispa *is due to the presence of apigenin and magnoflorine. The high antioxidant activity in* Tabernaemontana corymbosa *extract is due to its high phenol contents. There were significant linear positive correlation (*$r=0.788$, $p<0.001$, $r^2=0.621$*) between the total phenolic content and DPPH free radical scavenging assay in the crude extracts of* Tinospora crispa *and* Tabernaemontana corymbosa. *Meanwhile, a significant moderate positive correlation was observed between the total phenolic content and ferric reducing power assay (*$r= 0.556$, $p<0.05$, $r^2= 0.309$*). However, there was no significant difference in the correlation coefficient of total phenolic content and metal chelating assay.*

Keywords: Antioxidant activity; Tabernaemontana corymbosa; Tinospora crispa*; total phenolic content*

ABSTRAK

Tinospora crispa *dan* Tabernaemontana corymbosa *telah digunakan dalam perubatan tradisi untuk merawat demam, diabetik, reumatisme dan sinusitis. Objektif kajian ini adalah untuk menilai aktiviti antioksida dalam* Tinospora crispa *dan* Tabernaemontana corymbosa. *Kehadiran apigenin dan magnoflorin telah dikesan menggunakan LCMS/MS di dalam* Tinospora crispa *(Patawali) manakala appararicine, voafinine, conodusarine, conodurine, voacamine dan voacangine telah dikesan di dalam* Tabernaemontana corymbosa *(Susur kelapa) ekstrak methanol. Ekstrak batang* Tinospora crispa *menunjukkan aktiviti antioksida yang tinggi di dalam asai DPPH penyah radikal, kuasa penurun dan logam pengkelat dalam aturan berikut (98.25%, 0.957, 81.97%) daripada ekstrak daun (90.04%, 0.652, 69.64%), batang (82.78%, 0.819, 36.70%) dan akar (63.25%, 0.469, 51.56%)* Tabernaemontana corymbosa. *Aktiviti antioksida yang tinggi di dalam ekstrak batang* Tinospora crispa *adalah disebabkan kehadiran apigenin dan magnoflorin. Aktiviti antioksida yang tinggi di dalam ekstrak* Tabernaemontana corymbosa *adalah kerana kandungan fenol yang tinggi. Terdapat korelasi linear positif yang signifikan (*$r=0.788$, $p<0.001$, $r^2=0.621$*) antara kandungan fenolik dan asai DPPH penyah radikal di dalam ekstrak mentah* Tinospora crispa *dan* Tabernaemontana corymbosa. *Sementara itu, korelasi positif sederhana yang signifikan ditunjukkan antara jumlah kandungan fenolik dan asai kuasa penurunan ferik (*$r=0.556$, $p< 0.05$, $r^2=0.309$*). Walau bagaimanapun, tiada perbezaan yang signifikan dalam pekali kolerasi jumlah kandungan fenolik dan asai pengkelat logam.*

Kata kunci: Aktiviti antioksida; Tabernaemontana corymbosa*; jumlah kandungan fenolik;* Tinospora crispa

INTRODUCTION

Medicinal plants have been used as a source of medicine with their own personal recipes, which have been passed from one generation to another. Plant-derived substances have recently become of great interest owing to their versatile application (Baris et al. 2006). Natural products present in the medicinal plants have been used as a source of drugs in the traditional medicine and some of them have been scientifically explored (Nascimento et al. 2006).

In recent years, the use of natural antioxidants has been promoted because of the concerns on the safety against synthetic drugs (Shahidi 2000). Antioxidants play an important role in protecting against cell damaged by reactive oxygen species and decreasing the adverse effects of these free radicals on normal physiological functions in humans. Phenolic compounds are the class of antioxidant agents, which can act as free radical terminators (Shahidi & Wanasundara 1992) and possess scavenging ability due to their hydroxyl group (Diplock 1997). The antioxidant

activity of phenolic is mainly due to their redox properties that allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. According to Rice-Evans et al. (1995), they may also have a metal chelating potential. The typical phenolic compounds that possess antioxidant activity have been characterized as phenolic acids and flavonoids (Kahkonen et al. 1999). The phenolic acids have been reported and implicated as natural antioxidants in vegetables, fruits and other plants such as vanillic acid, ferrulic acid and caffeic acid which were widely distributed in the plant kingdom (Larson 1988).

The *Tabernaemontana corymbosa* (Susur kelapa) belongs to Apocynaceae family and commonly cultivated for its ornamental and fragrant flower. It has been used traditionally to treat cancer (Hsu 1967), wound and inflammations (Atta-Ur-Rahman et al. 1985), uterine stimulant (Da Sil Va et al. 1984), antiplasmodic and hypotensive (Dhar et al. 1968). The *Tinospora crispa* (Patawali) of Menispermaceae family are used in the form of decoction to treat cholera, diabetes (Burkill 1935; Hamdan Noor & Ascroft 1989; Perry 1980; Umi Kalsom et al. 1995) and hypertension (Nor Aziyah et al. 2001; Shahimi & Mohsien 1979).

In this present paper, we report the antioxidant activity of *Tabernaemontana corymbosa* and *Tinospora crispa* using DPPH, ferric reducing and metal chelating assay. The correlation between the total phenolic content (TPC) and antioxidant activity of *Tabernaemontana corymbosa* and *Tinospora crispa* was also investigated.

Materials and Methods

Plant Materials

The *Tabernaemontana corymbosa* was collected at Selandar, Melaka and *Tinospora crispa* at Jelebu, Negeri Sembilan. The leaves, stems and roots of the *Tabernaemontana corymbosa* and the stems of *Tinospora crispa* were washed and cut into small pieces. Then the samples were dried in oven at 40ºC for 3 days and ground into fine powders for extraction purposes.

Preparation of Plant Crude Extraction

Powdered samples (20 g) were extracted with 200 mL petroleum ether, chloroform, methanol and water. The mixture was placed in environmental shaker for 3 days. Then it was filtered and the filtrate was dried at 40ºC using rotatory vacuum evaporator. The dried crude extracts were weighed and kept in sample bottle at -20ºC until further used.

Separation, Detection and Identification of Phytochemical Compounds

The thin layer chromatography was carried out to detect the chemical compounds present in the crude extract of *Tabernaemontana corymbosa* and *Tinospora crispa*. The extract sample was placed as a band with capillary tube on Silica gel 60 F_{254} – precoated TLC plate of size 20 cm × 20 cm. The plate was allowed to develop a separation chromatography using solvent chloroform; acetone: methanol: 25% ammonia (20:6:3:1). The developed plate was dried and viewed under UV-light at 254 nm. Then, it was sprayed with dragendroff's, vanillin-sulphuric acid and anisaldehyde-sulphuric acid reagents to detect the presence of alkaloids, terpenoids and phenolic compounds, respectively. The chemical constituents of the methanol extract were determined using LCMS/MS with known standard references using ionisation mode: Positive and negative; Column: Phenomenex aqua C18 – 50 mm × 2.0 mm × 5 uM; Buffer: Water and acetonitrile with 0.1% formic acid and 5 mM ammonium formate. 1.0 mL of sample extracts was diluted five times with methanol and filtered with 0.2 uM nylon filter prior to analyses. The mass fragmentations were based on journal references and using standards references.

Total Phenolic Content (TPC)

The total phenolic content was measured using Folin-Ciocalteu method as described by Amin et al. (2004) with slight modification. All samples and readings were prepared and measured in triplicate. Gallic acid was used as standard at concentration ranging from 0.01 mg/mL to 0.05 mg/mL were prepared by diluting the stock solution with distilled water. The extract was prepared at concentration of 1 mg/mL. Briefly, 100 µL of extract was transferred into a test tube and 0.75 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) was added and mixed. The mixture was allowed to stand at room temperature for 5 min. Then, 0.75 mL of 6% (w/v) sodium carbonate was added to the mixture and mixed gently. After standing at room temperature for 90 min, the absorbance was read at 725 nm using UV/Vis spectrophotometer. The standard calibration curve of gallic acid (0.01 mg/mL - 0.05 mg/mL) was plotted to get the r^2 value and linear equation of gallic acid.

Determination of Antioxidant Assays

2,2,-Diphenyl-1-Picryl-Hydrazyl (DPPH) Free Radical Scavenging Assay

The DPPH free radical scavenging activity was determined using the method as described by Blois (1958) with some modification. Ascorbic acid was used as positive standard (0.0025 mg/mL, 0.0125 mg/mL, 0.0375 mg/mL, 0.125 mg/mL, 0.25 mg/mL and 0.5 mg/mL). Briefly, 500 µL of 0.1 mM of DPPH in methanol was added to 4 mL of samples crude extracts. After 30 min, the absorbance was measured at 517 nm. The percentage of inhibition was calculated and the graph was plotted to determine the IC_{50} value.

Reducing Power Assay

The reducing power was determined according to the method of Oyaizu (1986) with slight modification. BHA was used as positive standard reference. The plant crude extract of different concentrations ranging from 0.0625 mg/

mL to 1 mg/mL were added to 2.5 mL of 2.0 M phosphate buffer (pH6.6) and 2.5 mL of 1% potassium ferricyanide. The mixtures were incubated at 50ºC for 20 min. Then 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 1000 rpm for 10 min. Aliquot (2.5 mL) of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. After 30 min, the absorbance was measured at 700 nm.

METAL CHELATING ASSAY

The metal chelating was estimated following the method of Dinis et al. (1994). EDTA was used as positive standard reference. The plant extracts (0.0625 mg/mL -1 mg/mL) was added to 0.05 mL of 2 mM $FeCl_2$. The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine and the mixture was shaken vigorously. After left standing at room temperature for 10 min the absorbance of the solution was then measured spectrophotometrically at 562 nm.

STATISTICAL ANALYSIS

The experimental results were mean (SD) of three parallel measurements. Statistical analysis was performed using SPSS 16.0 software. Significant differences between samples were analyzed using analysis of variance (ANOVA) with Banferonni adjustment. Pearson's correlation was used to determine the correlation of data between DPPH free radical-scavenging activity, reducing power assay and metal chelating assay on total phenolic content. The values for $p<0.05$ were regarded as significant and $p<0.01$ as highly significant.

RESULTS AND DISCUSSION

The presence of phenolics and terpenoids were detected with phenol and vanillin sulphuric acid reagent, respectively. The separation and detection of the stem extract of *Tinospora crispa* with LCMS/MS showed the presence of apigenin (Figure 1) and isoquinoline alkaloid, magnoflorine (Figure 2). The detection of apigenin validated the discovery of apigenin by Umi Kalsom and Noor (1995). Magnoflorine has also been detected in *Tinospora cordifolia*. In the leaves extract of *Tabernaemontana corymbosa*, it showed the presence of appararicine (Figure 3) and voafinine (Figure 4) whereas in the stem conodusarine (Figure 5) was detected. The root extract of *Tabernaemontana corymbosa*, showed the presence of conodurine (Figure 6), voacamine (Figure 7) and voacangine (Figure 8). All of the compounds detected were identified with known standard referenced. Thus, from these results it can be postulated that the high antioxidant activity in the *Tinospora crispa* was attributed to the presence of apigenin, magnoflorine whereas in *Tabernaemontana corymbosa* was due to the presence of appararicine, voafinine and conodusine.

TOTAL PHENOLIC CONTENT (TPC)

The contents of total phenols were measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: $y=0.0066x + 0.0102$, $r^2=0.9954$). Table 1 shows that the highest total phenolic content was observed in the leaves extract of *Tabernaemontana corymbosa* with 124.61±2.17 GAE/mg followed by the root extract with 94.67±1.41 GAE/mg, stem extract with 76.64±3.11 GAE/mg and stem extract of *Tinospora crispa* with 64.67±1.64 GAE/mg. Methanol is the most suitable solvent in the extraction of phenolic compounds due to its ability to inhibit the reaction of polyphenol oxidase that causes the oxidation of phenolic and its ease of evaporation compared with water (Yao et al. 2004). Most of the values were significantly different at $p<0.05$. The results suggested that most of the extracts varied

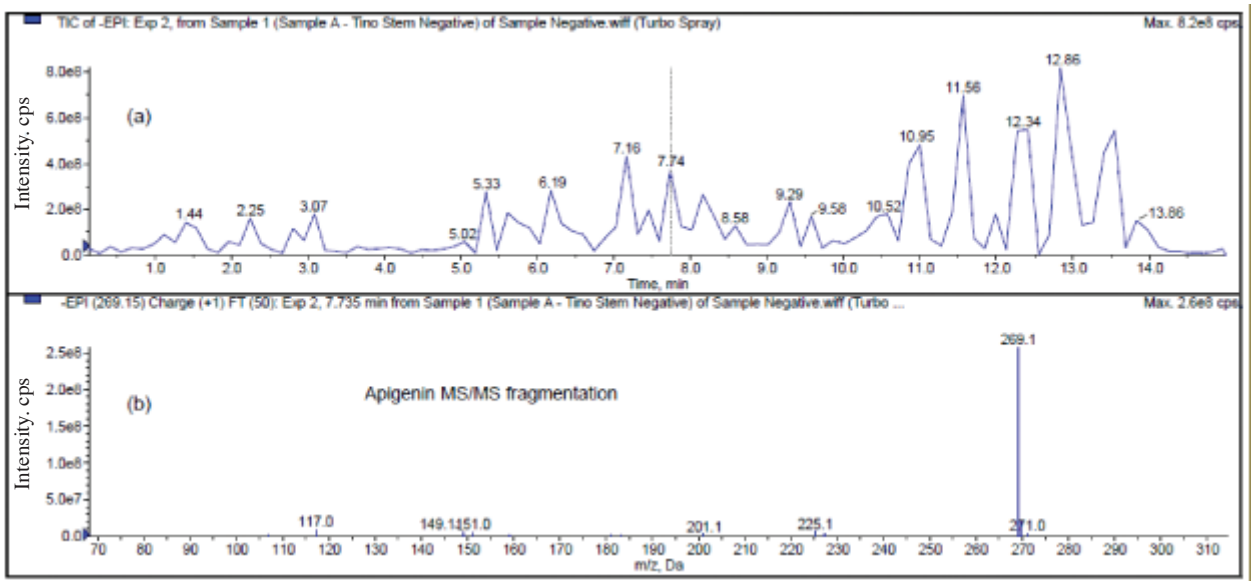

FIGURE 1. (a) LCMS profile of the stem extract of *Tinospora crispa* and (b) MS/MS fragmentation of apigenin

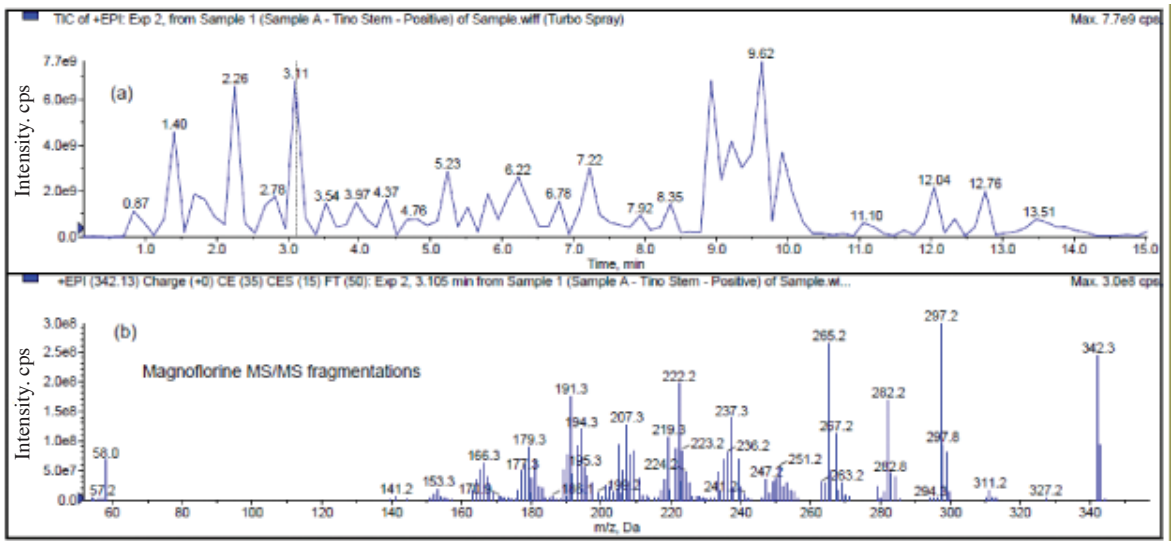

FIGURE 2. (a) LCMS profile of the stem extract of *Tinospora crispa* and (b) MS/MS fragmentation of magnoflorine

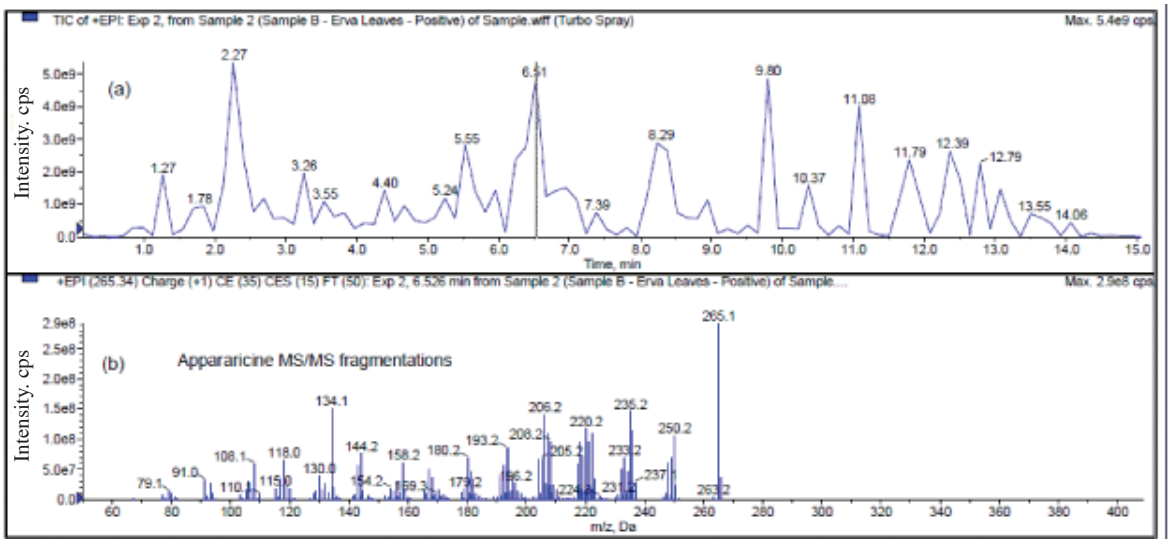

FIGURE 3. (a) LCMS profile of the leaves extract of *Tabernaemontana corymbosa* and (b) MS/MS fragmentation of appariricine

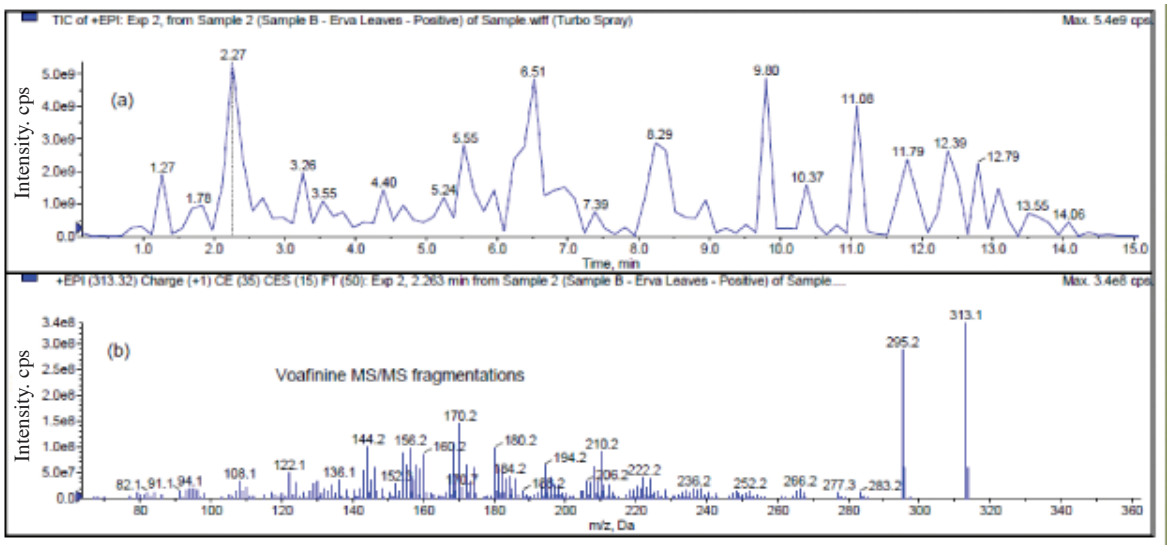

FIGURE 4. (a) LCMS profile of the leaves extract of *Tabernaemontana corymbosa* and (b) MS/MS fragmentation of voafinine

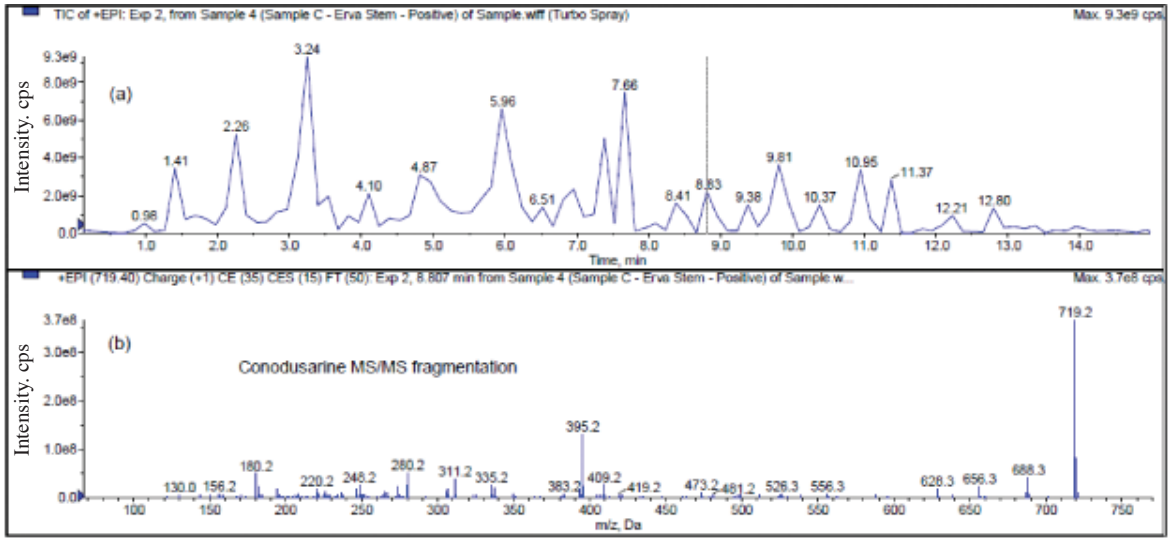

FIGURE 5. (a) LCMS profile of the stem extract of *Tabernaemontana corymbosa* and (b) MS/MS fragmentation of conodusarine

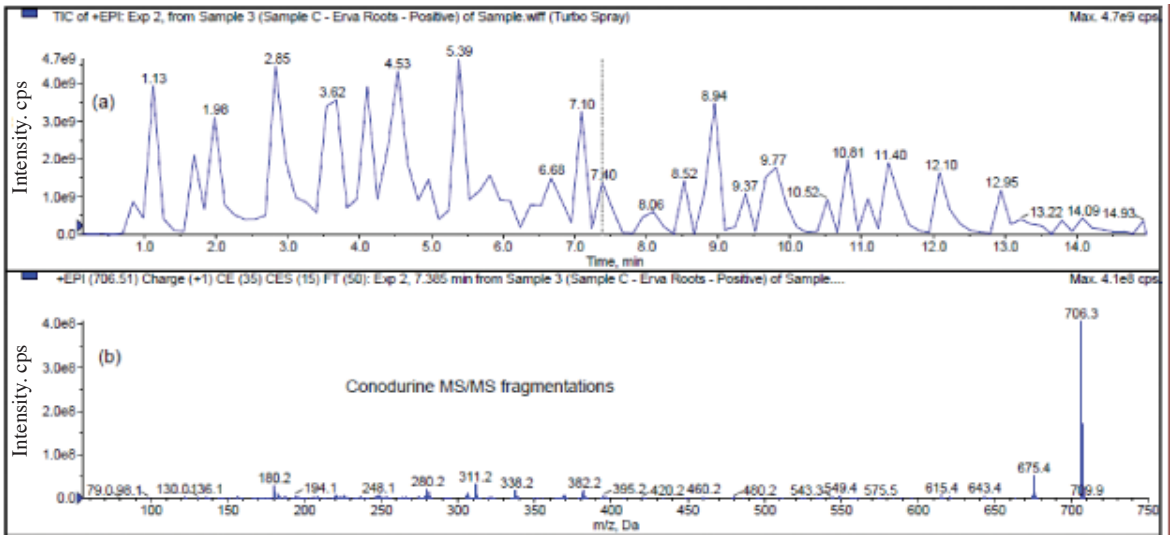

FIGURE 6. (a) LCMS profile of the roots extract of *Tabernaemontana corymbosa* and (b) MS/MS fragmentation of conoduranine

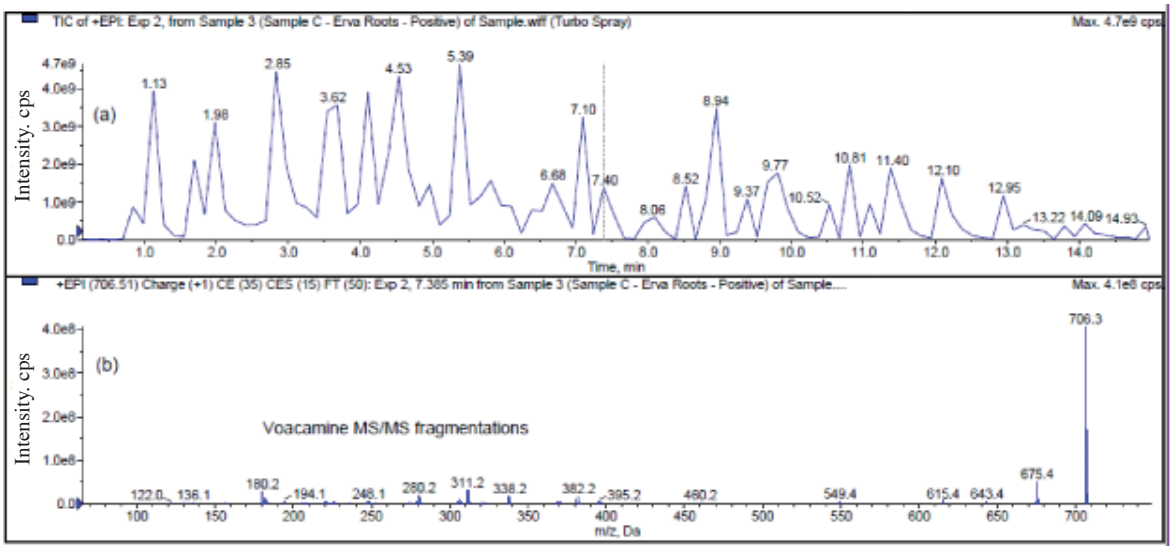

FIGURE 7. (a) LCMS profile of the roots extract of *Tabernaemontana corymbosa* and (b) MS/MS fragmentation of voacamine

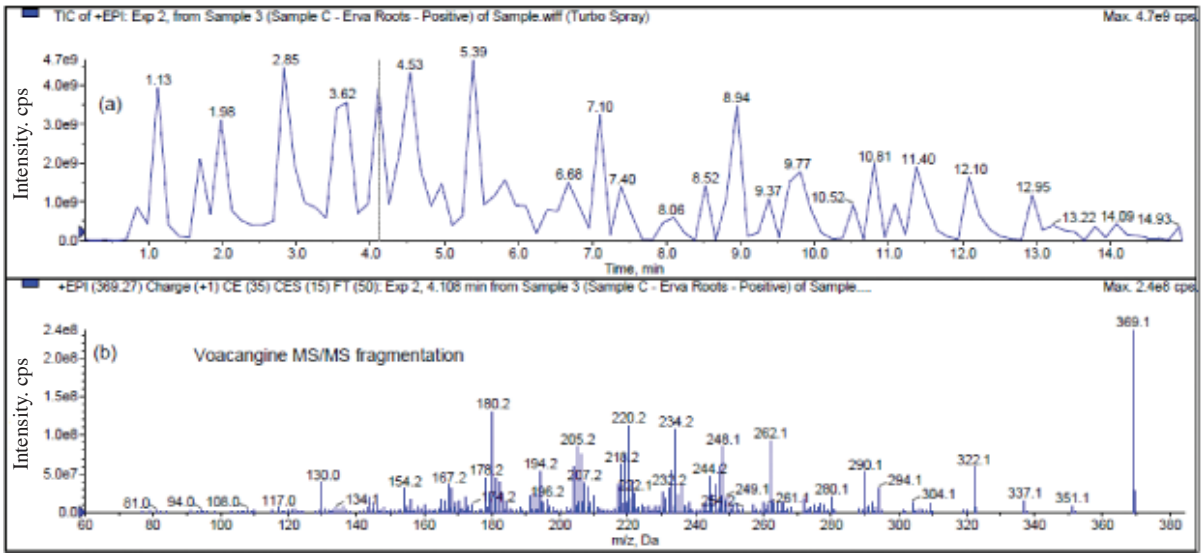

FIGURE 8. (a) LCMS profile of the roots extract of *Tabernaemontana corymbosa* and (b) MS/MS fragmentation of voacangine

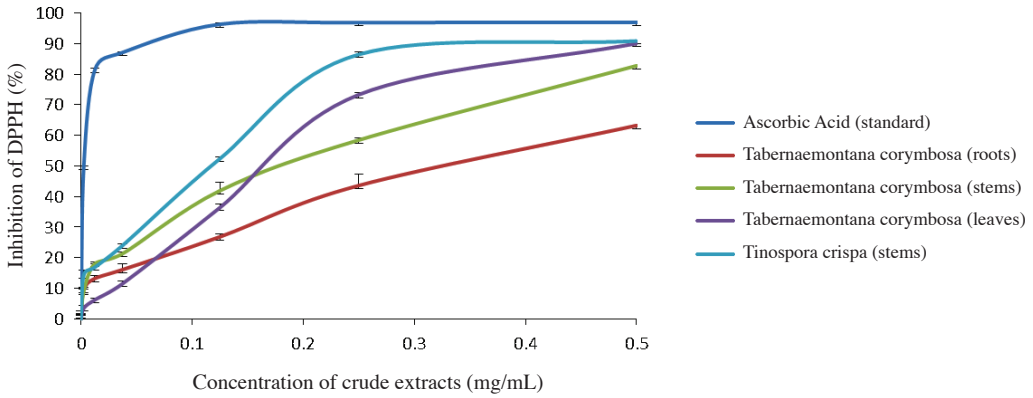

FIGURE 9. Dose-response curve of DPPH radical scavenging assay of the crude extracts

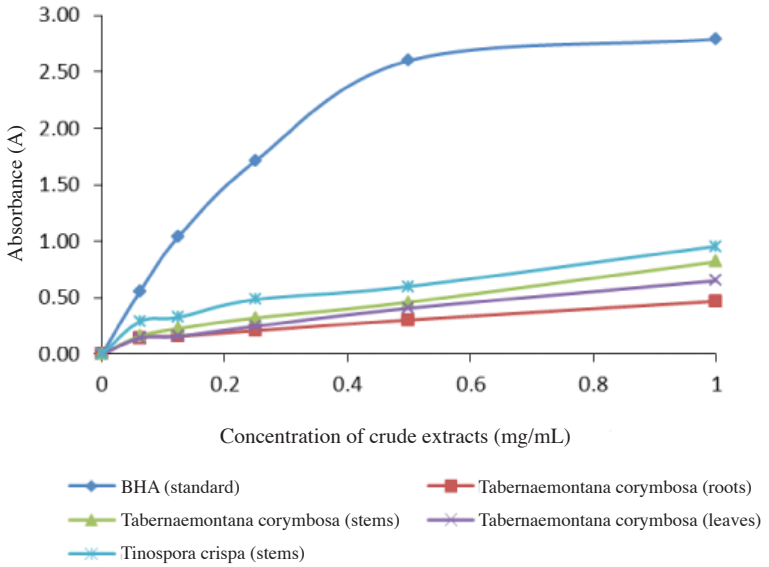

FIGURE 10. Dose-response curve of reducing power assay of the crude extract

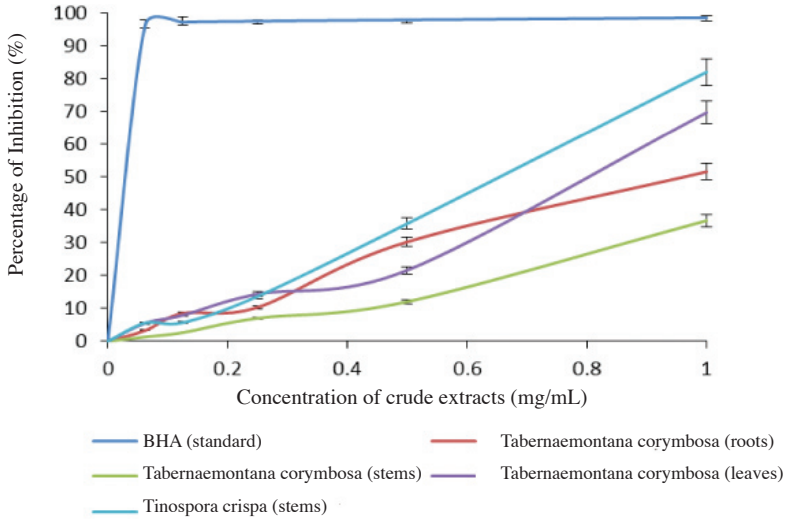

FIGURE 11. Dose-response curve of metal chelating assay of the crude extracts

TABLE 1. Total phenolic content of the extract of *Tinospora crispa* and *Tabernaemontana corymbosa*

Plants sample	Total phenol content (GAE/mg)
Tinospora crispa stem	64.67±1.64[a]
Tabernaemontana corymbosa leaves	124.61±2.17[b]
Tabernaemontana corymbosa stem	76.64±3.11[c]
Tabernaemontana corymbosa root	94.61±1.41[d]

Different letters indicate significant difference at $p<0.05$

TABLE 2. The correlation of antioxidant assays and the total phenol contents in *Tinospora crispa* and *Tabernaemontana corymbosa*

		Antioxidant assays			
		TPC	DPPH	Ferric reducing	Metal chelating
TPC	Pearson Correlation Sig. (2-tailed)	1.000	0.788**	0.556	0.358*
DPPH	Pearson Correlation Sig. (2-tailed)	0.788**	1.000	0.751**	0.282
Ferric reducing	Pearson Correlation Sig. (2-tailed)	0.556*	0.751**	1.000	0.281
Metal chelating	Pearson Correlation Sig. (2-tailed)	0.358	0.282	0.281	1.000

** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

Key:

TPC - Total Phenol Content
DPPH - DPPH Radical Scavenging Activity
METAL - Metal Chelating Assay
REDUCING - Reducing Power Assay

significantly from one extract to another in term of TPC. These results gave an indication that the total phenol might also contributed to the antioxidant activity in *Tinospora crispa* and *Tabernaemontana corymbosa*.

DPPH RADICAL SCAVENGING ASSAY

The DPPH radical scavenging assay is a decolonization assay that will measure the capacity of antioxidants to directly scavenge DPPH radicals by measuring its absorbance with spectrophotometer at 517 nm. In the presence of R-H (hydrogen/electron donating compound) or antioxidant, DPPH radical will be reduced to its non-radical form giving rise to the color ranging from yellow to colorless. Its absorption intensity also decreased according to the number of electron captured (Markowicz Bastos et al. 2007).

Ascorbic acid was used as a positive standard and suitable for DPPH radical scavenging assay (Kim et al. 2002). The IC_{50} for ascorbic acid was 0.004 mg/mL with percentage of inhibition of 96.97% at 0.5 mg/mL concentration.

The DPPH radical scavenging activity or percentage of inhibition of free radical of the leaves, stem, root extracts

of *Tabernaemontana corymbosa* as well as the stem extract of *Tinospora crispa* increased with the increasing concentrations of the extracts (Figure 9). The stem extract of *Tinospora crispa* showed the highest DPPH inhibition at 98.85% (IC_{50}=0.118 mg/mL) followed by leaves extract of *Tabernaemontana corymbosa* at 90.04% (IC_{50}=0.162 mg/mL), stem extract at 82.78% (IC_{50}=0.176 mg/mL) and root extract at 63.25% (IC_{50}=0.34 mg/mL) at 0.5 mg/mL concentration. The high antioxidant activity of the stem extract of *Tinospora crispa* is most probably due to the presence of apigenin and magnoflorine as its possess hydroxyl group that donates the electron to reduce the DPPH radicals. Rackova (2004) has reported that magnoflorine isolated from *Mahonia aquifolium* showed high antiradical activity toward DPPH radical. Magnoflorine has also been shown to have high antioxidant activity in the seed of *Xanthoxylum piperitum* (Hisatomi et al. 2000) and in the root of *Tinospora cordifolia* (Rekha & Veena 2011). Similarly, Cavin et al. (1998) has shown that the compounds N-cis-feruloyltyramine, N-trans-feruloyltyramine and secoisolariciresinol exhibited antioxidant and radical scavenging properties towards 2,2,-diphenyl-1-picrylhydrazyl (DPPH). The high antioxidant activity of the leaves extract of *Tabernaemontana corymbosa* is probably due the presence of high phenolic content since phenolic compounds are effective as hydrogen donors and exhibit antioxidative activity (Hatano et al. 1989; Rice-Evan et al. 1995). Thus, therapeutic properties of *Tabernaemontana corymbosa* may be possibly attributed to the phenolic compounds present.

Cavin et al. (1998) who found the presence of three compounds identified as N-cis-feruloyltyramine, N-trans-feruloyltyramine and secoisolariciresinol which exhibited antioxidant and radical scavenging properties towards 2,2,-diphenyl-1-picrylhydrazyl (DPPH).

REDUCING POWER ASSAY

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir et al. 1995) and one of the methods to evaluate antioxidant activity of plants. The method works based on mechanism of reduction of Fe^{3+} from ferricyanide complex to ferrous form (Fe^{2+}) resulting in an intense blue chromogen which can be monitored by measuring the change in absorbance. In the reducing power assay, the change of yellow color of the test solution to blue-green depends on the strength of the reducing power of each extract. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

In this study, butylated hydroxyanisole (BHA) was used as a positive standard due to the greatest ability to reduce the rate at which other substances undergo oxidation. Therefore, it is a good antioxidant compound. The reducing power of BHA increased with the increasing concentration. At concentration 1 mg/mL, BHA had the highest ability to reduce the Fe^{3+} with absorbance at 2.794. High absorbance indicates greater reducing power. The reducing power for each extract exhibited low reducing activity in comparison with reference standard BHA. The stem extract of *Tinospora crispa* showed the highest absorbance at 0.957 followed by *Tabernaemontana corymbosa* stem extract at 0.819, leaves extract at 0.652 and root extract at 0.469 at the concentration of 1 mg/mL (Figure 10). The high antioxidant ability of the stem extract of *Tinospora crispa* to reduced ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) form is corresponding to the high in DPPH inhibition. This suggested that the apigenin and magnoflorine present in the stem extract of *Tinospora crispa* have the ability to donate electron and reduced the ion (Fe^{3+}) to ferrous ion (Fe^{2+}) form. Meanwhile the ability of extract of leaves, stem and root of *Tabernaemontana corymbosa* were due to the presence of high content of phenol as compared with *Tinospora crispa*.

METAL CHELATING ASSAY

The metal chelating activity is significant as it reduces the concentration of the catalysing transition metal in lipid peroxidation through the Fenton reaction (Hseu et al. 2008). The transition metals such as ion can stimulate the lipid peroxidation of generating hydroxyl radicals through Fenton reaction and accelerate lipid peroxidation into peroxyl and alkoxyl radicals eventually drive the chain reaction of lipid peroxidation (Zhao et al. 2006). Chelating agents may inhibit radical generations by stabilizing transition metals, consequently reducing free radical damage. Besides, some phenolic compounds exhibit antioxidant activity through the chelation of metal ions (Zhao et al. 2008).

In this work, EDTA (ethylenediamine tetraacetic acid) was used as a positive standard reference due to its strong metal chelator properties (Gülçin et al. 2005). Its structure consists of nitrogen atom and short chain carboxylic group. As shown in Figure 11, the percentage of inhibition of ferrozine-Fe^{2+} complex formation of EDTA increased with the increasing concentration. All the concentrations tested in this assay exhibited significantly high inhibition of ferrozine-Fe^{2+} complex. At concentration of 1 mg/mL, EDTA obtained the highest percentage of inhibition at 98.51%.

The absorbance of Fe^{2+}-ferrozine complex was decreased dose dependently where the activity was increased on increasing concentration from 0.0625 mg/mL - 1 mg/mL. In comparison with all the extracts of both plants, stem extract of *Tinospora crispa* showed high inhibition metal chelating at 81.97%, followed by *Tabernaemontana corymbosa* leaves extract at 69.64%, root extract at 51.56% and stem extract at 36.7% inhibition Fe^{2+}-ferrozine complex at 1.0 mg/mL concentration. This indicated that the extract can interfere with the formation of ferrous and ferrozine complex, thus suggesting the potential of the extract to chelate and capture ferrous ion before ferrozine. The highest activity of metal chelating of the stem extract of *Tinospora crispa* which is corresponding to high activity in reducing power assay and DPPH assay showed an indication that it is due to the

present of apigenin and magnoflorine. Hung et al. (2007) have shown that magnoflorine is able to prevent oxidation of various LDL via copper-mediated (Cu^{2+}) oxidation of LDL. This provide evidence for the high chelating activity *Tinospora crispa* stem extract.

CORRELATION OF TOTAL PHENOLS CONTENT AND ANTIOXIDANT ASSAYS

The total phenols contents are closely related with antioxidant activity. The results in Table 2 shows that there are significant good linear positive correlation (r=0.788, p<0.001, r^2=0.621) between the total phenols content and DPPH free radical scavenging assay in the extracts of both plants. In addition, significant moderate positive correlation was observed between the total phenols content and reducing power assay (r=0.556, p<0.05, r^2=0.309). However, there is no significant difference between total phenols content and metal chelating (p>0.05). This indicated that there are satisfactorily good relationship between phenols content and antioxidant activity in *Tinospora crispa* and *Tabernaemontana corymbosa* extract. Velioglu et al. (1998) have reported a strong relationship between the total phenolic content and antioxidant activity in certain plant products.

CONCLUSION

In the stem extract of the *Tinospora crispa*, apigenin and magnoflorine was detected by LCMS/MS whereas in the extracts *Tabernaemontana corymbosa* appararicine, voafinine, conodusarine, conodurine, voacamine and voacangine were detected. The stem extract of *Tinospora crispa* showed the highest antioxidant activity in DPPH, reducing power and metal chelating assay than in *Tabernaemontana corymbosa extract*. The high antioxidant activities in *Tinospora crispa* are mostly due to the presence of apigein and magnoflorine as these compounds possess antioxidant properties. There was a positive correlation between the total phenolic content and antioxidant activity in DPPH, reducing power and metal chelating in both extract of *Tinospora crispa* and *Tabernaemontana corymbosa*. Thus, these findings provided scientific evidence that *Tinospora crispa* and *Tabernaemontana corymbosa* possesses antioxidant properties that contribute to its medicinal use.

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Institute of Biological Sciences
Faculty of Science
University of Malaya
50603 Kuala Lumpur
Malaysia

*Corresponding author; email: jamal@um.edu.my

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